Establishment and mass breeding of *Phlebotomus papatasi* (Diptera: Pshychodidae) in Orzouieh county in Kerman Province, Southeast of Iran

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ABSTRACT

Background & objectives: Sandflies are important carriers of *Leishmania* parasites. Leishmaniasis, spread by sandfly vectors, is a major public health problem worldwide particularly in the tropics and the subtropics. The parasite transmission capacity of vectors, life cycle of parasites in the body of vectors, disease transmission, and the physiology and behavior of carrier vectors can be extensively studied by establishing insect colonies in the insectarium. In addition, response of vectors to repellents and insecticides, mating behaviors, blood feeding habits, interaction between parasites, and vectors, and taxonomic studies can be investigated by establishing insect colonies. The goal of the present study was to establish a colony of *Phlebotomus papatasi* in the insectarium in a zoonotic cutaneous leishmaniasis endemic area in Kerman Province of Iran.

Methods: The Killick-Kendrick and Killick-Kendrick method (1991) was used for individual rearing and the volf and volfa (2011) method was used for mass rearing of the collected sandflies. The larvae were fed by a diet with liver powder, which is the recommended diet for *Phlebotomus papatasi* larvae. Adult sandflies were allowed to feed on BALB/c mice blood in the laboratory.

Results: Eighty sandflies specimens (75 female + 5 male) were collected with aspirator and reared for f1 and f2 generations in the laboratory.

Interpretation & conclusion: Phlebotomus sandflies were colonized in laboratory condition. Then sandflies successfully maintained for the first time as a laboratory species in Kerman Province.

Key words Leishmaniasis; sandfly; rearing; Kerman; Iran

INTRODUCTION

Leishmaniasis is an important protozoan disease in humans created by more than 20 species of *Leishmania.* These parasites are transmitted by more than 90 sandfly species[1]. About 927–1000, species sandflies have been identified worldwide, and they commonly inhabit tropical and subtropical regions of the world[2–4]. Sandflies are important carriers of *Leishmania* parasites. Leishmaniasis, spread by sandfly vectors, is a major public health problem worldwide especially in the subtropics and the tropics[5]. The importance of laboratory colonization and mass rearing of Phlebotomine sandflies was highlighted by Safyanova[6]. Laboratory species of Phlebotomine sandflies are important experimental models for studying the biology and behavior of the vectors and their interaction with disease agents. In addition, new experiments for vector control methods can be tested on laboratory species[6]. There are currently 90 colonies established from 21 distinct Phlebotomine sandflies species located in 35 laboratories in 18 countries throughout the world, which are registered in the world list of sandfly colonies[7]. The capacity of vectors to transmit parasites, the life cycle of parasites in the body of a carrier, disease transmission capacity, and the physiology and behavior of vectors can be extensively investigated using laboratory models of sandfly species. In addition, response of vectors to repellents and insecticides, mating behaviors, blood-feeding habits, the interaction between parasites and carriers, and taxonomy can be studied by establishing laboratory colonies of vectors[8–9]. Kerman Province is one of the important foci of cutaneous leishmaniasis in Iran. Cutaneous leishmaniasis (CL) has been reported from southeastern parts of the country such as Kerman, Bam, Rafsanjan, Jiroft, Baft, Shahr-e-Babak and Sirjan in Kerman Province. The purpose of establishing laboratory colonies of *Phlebotomus papatasi* in the world is to study the potential of parasite transmission, life cycle *of Leishmania* parasite, mode of transmission and its dynamics. Possibility of using salivary gland secretions in preparing a vaccine against leish-

maniasis, study of biology, physiology and vector behavior, evaluation of repellents and insecticides, evaluation of nets impregnated with insecticides, testing of sorbents, mating behaviors, blood-feeding method, investigation of parasite-vector interaction, taxonomic studies and study of virus-vector interaction is required.

The goal of the present study was to establish a colony of *Phlebotomus papatasi* in the insectarium in a zoonotic cutaneous leishmaniasis (ZCL) endemic area in the Kerman Province of Iran. This comprehensive study was conducted for the first time in Kerman Province due to the existence of indigenous centers of rural and urban cutaneous leishmaniasis in this province. The results of this study will be a positive step for further research on these important vectors of cutaneous leishmaniasis and will be applied by researchers and technologists for future investigations.

MATERIAL & METHODS

Collection site

Orzouieh County is one of the endemic foci of cutaneous leishmaniasis (CL) in Kerman Province in Iran[10]. In this experimental study, sandflies were collected from Takht-e-khajeh village in Orzouieh County in the southwestern part of Kerman Province in southeast of Iran (Fig. 1). This county has recently been separated from Baft and consists of four main areas viz., Vakilabad, Sultanabad, Dolatabad and Shahmaran.

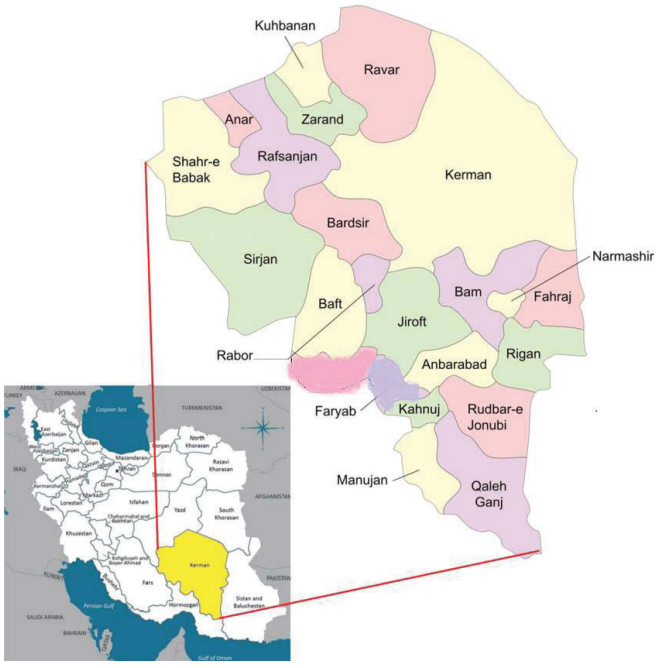

Fig. 1: Study region in Orzouieh rural district,Takht-e-Khajeh village, Baft County, Kerman Province, southeast Iran, 2020–2021.

Inclusion and exclusion criteria

Inclusion criteria was *Phlebotomus papatasi* san fly caught from Orzouieh center of Baft city of Kerman province. Exclusion criteria was other species of sandflies caught from this area.

Collection of wild sandflies for initiating colony

Killick-Kendrick and Killick-Kendrick[11] indicated, "The establishment of sandflies colony is much harder than the keeping of formerly established colony". In this research, we discuss the methods for setting up a new colony and processing with a focus on cutaneous leishmaniasis in southeast of Iran.

Collection of sandflies by aspirators (hand catch method)

Sandflies were collected from outdoor habitats before sunrise using the hand catch method (mouth aspirators) and at sunset by hand catch method via car trap at 8.00 pm to 12.00 pm. Collected sandfly specimens were transferred to breeding cages and transported alive to the insectarium of Medical School, Kerman University of Medical Sciences for rearing. The breeding cages contained wet clothes and 20% sucrose-impregnated cotton pads for feeding the flies. The cages were transported in a nylon cloth preventing temperature and humidity changes.

Processing of wild population of sandflies collected in the field

The colonies of sandflies were maintained in the insectarium in an optimum temperature at 25°C to 28°C and 14:10 (light: dark) photoperiod. Appropriate photoperiod is probably one of the most important factors in the laboratory colonization of insects; 14:10 (light: dark) is used in many laboratories (Table 1).

A high humidity (70–95%) is achieved with packaging the breeding cage inside a plastic bag with a wet paper towel or cotton cloth inside. Two methods are commonly used for laboratory colonization of insects; the Killick-Kendrick and Killick-Kendrick method (1991) for individual rearing of insects[12], and the volf and volfa 2011 method[13] for mass rearing of insects[14].

For breeding of the sandflies, we transferred one female and male into a pot for mating and oviposition as described by Modi & Tesh (1983)[14]. The wild sandflies were fed with 50% honey solution and saturated sucrose. Breeding containers were examined daily for hatching. After hatching of the eggs, the newly hatched larvae were fed standard food; a mixture of chewing food powders and rabbit feces in equal amounts and if necessary, as a dietary supplement, liver powder was added to the basic food[8, 9, 15–16]. The larvae at different development stages were fed

Table 1. Sandfly colonies were maintained in the insectarium of Leishmaniasis Research center of Kerman University of Medical Sciences in 2020–2021.

Genus	Species	Origin	Maintained since	Study Year	Egg	Emerged adult	Duration of generation
Phlebotomus	*papatasi*	Orzouieh County Kerman	2020	2020	115±123.86	56 (31 female + 25 male)	28.87±24.40 (28.87-52)

the same diet as described above. For preventing fungal contamination during transfer of larvae into new pots, a small amount of autoclaved soil was added to the diet of the larvae.

Identification and detection of sandfly species is done in separate stages after death. After transparency, the sandfly detects assembly of the material in a Pouri environment, based on morphological features and using valid diagnostic keys[7, 15–16].

The sandfly pupae in the pots were transferred into a new cage containing a damp cloth and 20% sucrose sugar solution with soaked in cotton pads as a food source. The pupae were reared as described by Killick-Kendrick *et al*.,[12]. Volf & Volfa in 2011[12] used three different pot sizes for mass rearing of sandflies. The smallest pot has a diameter of 6 cm and can accommodate up to 20 pregnant sandflies and the largest pot (diameter 14 cm) can accommodate 100–150 pregnant female sandflies. The size of the pot is important mainly for the growth and development of larvae.

Ethical statement

The ethics committee of Kerman University of Medical Sciences, Iran, approved this study with ethical code number IR.KMU.REC.1398.283.

RESULTS

The collected sandfly species from Orzouieh county, Thakht-e-Khajeh village were identified as *Phlebotomus papatasi.* Sandflies were allowed to feed on BALB/c mouse blood. 80 sand flies (75 female + 5 male) were collected with mouth aspirator and reared for f1 and f2 generation in the laboratory (Fig. 2). Life cycle of sandflies in the laboratory included eggs, larvae 1, 2, 3, 4, pupae, and adult stages (Fig. 3).

DISCUSSION

This comprehensive study was conducted for the first time in Kerman province due to the existence of endemic centers of rural and urban cutaneous leishmaniasis in this province according to a written plan to achieve the desired goals. Kerman Province is one of the important foci of ACL and ZCL in the country and there is a need to establish a sandfly colony in this area which can be useful for vector control, disease transmission, and parasite transmission studies. We hope that the results will be a positive step for further research on this important vector of cutaneous leishmaniasis.

Studying the biological characteristics of laboratory species of sandflies will provide better insights into their life cycle. In addition, laboratory colonization of sandflies will provide an opportunity to study parasite and invertebrate host interactions, susceptibility to insecticides and vector control methods[16]. However, the establishment of insect colonies of sandflies from a wild population is a difficult, time-consuming, uncertain, and challenging task[17]. Another major problem of laboratory colonization of sandflies is a genetic bottleneck that causes colony collapse after several generations[18]. An earlier attempt to colonize *P. papatasi* in Iran in 1968 was not successful[19]. In 2007, Yaghoobi *et al*., succeeded in establishing colonies of this species for several generations in Iran[8]. In the present study, the life-cycle duration was (28–52) days in the first generation (F1). In the study Yaghoobi *et al*., the durations of egg to female adult and egg to male adult were (34.8±17.2) days and (41.3±4.1) days, respectively[8]. In this study, the main problem encountered in the larval

Fig. 2: Sandflies blood feeding on anesthetized BALB/c mice in the laboratory, 2020–2021.

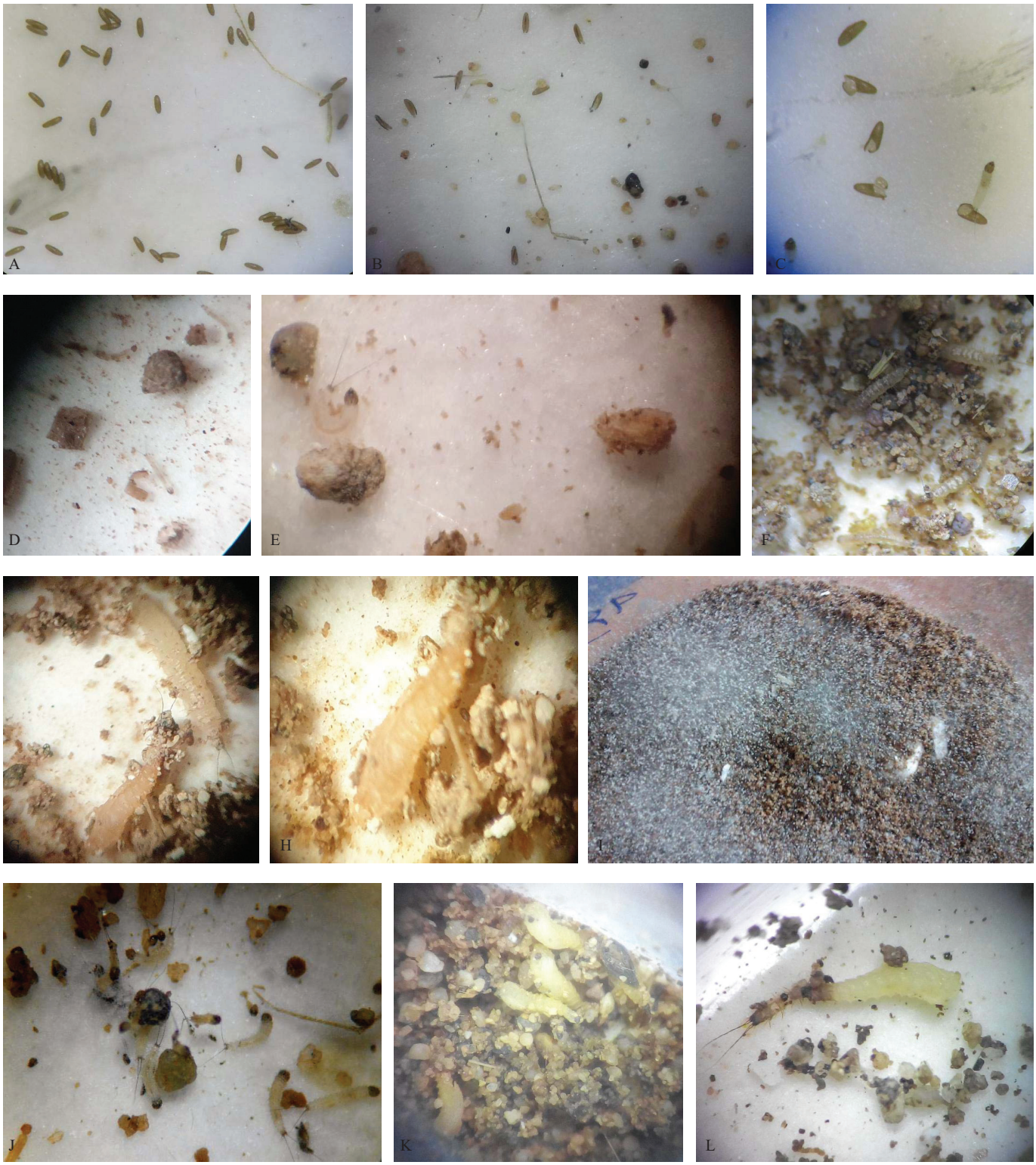

Fig. 3: Life cycle of *Phlebotomus papatasi*: A. eggs; B. L1, (1st instar larvae); C. Hatched eggs; D. L2 (2nd instar larvae); E. L3 (3rd instar larvae); F. L3 and L4 (3rd instar larvae, 4nd instar larvae); G. L4 (4th instar larvae); H. early stage of pupa; I. Fungi contamination in rearing pots and J. cannibalism in larvae, K. pupa few hours before emergence; L. 4th instar exuvium at the caudal end of the puparium (pupae in last stage); in the insectarium of Leishmaniasis Research center of Kerman University of Medical Sciences in 2020–2021.

stage was fungal contamination. Similar to previous studies, growth of fungi had harmful effects on instar larvae and eggs[9]. To reduce the effect of fungal contamination, we added autoclaved soil to the diet of the larvae in pots.

Excessive nutrition is also one of the major causes of infection during insects rearing. This can be controlled by eliminating uneaten foods[19] or by introducing sufficient larvae into each pot to prevent over-feeding. It is also possible to prevent fungal growth by using a sterile or natural feeding mixture. Between operations, the tools should be cleaned with warm water to prevent transfer of harmful fungi and mites from pot to pot[8, 9]. Cannibalism is a recurring behavior in animals and plays an important role in their population dynamics[20]. Killick-Kendrick studies indicated that fundamental problems in breeding of Phlebotomine sandflies are extreme larval mortality affected by fungal growth, unsuitable diet or humidity, bacterial disease, and cannibalism[12, 18].

The weak and strong points of our study included (1) weakness of studying a small number of san flies caught from the field., (2) the strengths of the study are the establishment of an insectarium for the first time in Kerman University of Medical Sciences and the breeding of sandflies for up to two generations.

LIMITATION

Insectarium space

In consultation with university officials and explaining the importance of the existence of the insectarium for specialized studies in the field of evaluation of insecticides, drugs, and vaccines, an attempt is made to provide and equip a suitable space for this work. (1) Collecting samples from the field: Due to the low abundance of *Phlebotomus papatasi* in the field and the presence of coronavirus pandemic, there may be problems in growing them in the laboratory. This problem is solved by increasing the number of sample cached from the field if necessary. (2) Problems arising from the specific ecological needs of sandflies: At the beginning of the establishment of the insectarium and laboratory breeding, the biological needs of *Phlebotomus papatasi* should be carefully studied by providing favorable conditions.

CONCLUSION

Considering that Kerman Province is one of the most important foci of urban and rural cutaneous leishmaniasis in Iran, there is an urgent need to set up and maintain the sandfly colony of *Phlebotomus sergeant* and *Phlebotomus papatasi*. Therefore, starting a new colony at an endemic site is a prerequisite for detailed studies on parasite-vector interactions, but it is a much more difficult step than routine maintenance of colonies that have already been established in the laboratory for several generations. San fly colonies can be used for biological studies, physiology, vaccine studies, and study of repellency effect of plant extracts.

Conflict of interest: None

ACKNOWLEDGEMENTS

Authors are sincerely grateful to Mr. Mehdi Afshari Pour, member of Dehbakri Health Center in Bam city, Kerman province, for his help and cooperation during the study. Authors also thank Ms. Abbasi, an employee of the health house in Takht-e-Khajeh village in Orzouieh county, Baft state, in Kerman province, for assisting in sand fly collection. The Vice Chancellor for Research, Kerman University of Medical Sciences, Iran, financially supported this project (No. 98000263).

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Received: 28 November 2021 *Accepted in revised form:* 19 January 2022